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Honest advertisement and mutual mate choice in the royal flycatcher (Onychorhynchus coronatus)

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HONEST ADVERTISEMENT AND MUTUAL MATE CHOICE IN THE ROYAL FLYCATCHER (*ONYCHORHYNCHUS CORONATUS*)

by

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Declaration of Co-Authorship

I hereby declare that this thesis incorporates material that is the result of joint research. Both data chapters are co-authored with my supervisor, Dr. Stéphanie Doucet, who supported my research, provided valuable feedback, helped with statistical analyses, and provided editorial input during the writing of both manuscripts. Chapter 2 was prepared as a manuscript, and has been submitted to Animal Behaviour for publication. Chapter 3 was also prepared as a manuscript for submission to The Auk.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from my co-author to include the above materials in my thesis. I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work, completed during my registration as graduate student at the University of Windsor.

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Abstract

Traditional studies of sexual selection have focused on elaborate male traits and female preferences. In many species, however, females exhibit some degree of ornamentation, and this pattern warrants examination. I investigated whether plumage colouration honestly signals quality and reproductive success in mutually ornamented royal flycatchers (*Onychorhynchus coronatus*), and to determine whether or not they exhibit mutual mate preferences by ornamentation. In Costa Rica, I monitored birds throughout the breeding season and collected data from breeding pairs. In the lab, I performed reflectance spectrometry on feathers and quantified aspects of quality. I found that both male and female plumage colouration honestly revealed aspects of quality and predicted reproductive success. However, I found no evidence that royal flycatchers mate assortatively. I conclude that plumage colouration may be an honest, sexually-selected trait in both sexes, but that assortative mating does not explain the evolution of female ornamentation in this species.

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CHAPTER 1: GENERAL INTRODUCTION

Sexual selection and honest advertisement

Several selection pressures, including natural selection, sexual selection, and social selection operate on individuals in every species, the consequence of which is the phenotypic and genetic diversity of life on this planet. Though the theory of natural selection, which posits the maintenance of traits that confer an advantage in survival was originally highly controversial (Darwin 1859), it was broadly accepted by the scientific community by the 1930's (Mayr 1982). In contrast, Darwin's theory of sexual selection, which proposes that individual competition for reproductive success drives the evolution of traits that confer greater mating success, did not receive serious attention until the 1970s (Darwin 1871, reviewed in Andersson 1994), with few exceptions (Fisher 1915, 1930). Traditionally, studies of sexual selection have focused almost exclusively on the dyad of conspicuous males and drab females. In these situations, sexual selection is often driven by male-male competition and female choice (Andersson 1994). While Darwin was the first to propose that female choice may be partly responsible for driving the elaboration of male traits (Darwin 1871), there are competing ideas on the origin of female preferences.

The Fisherian theory of runaway selection states that arbitrary female preference for a particular male trait leads to the production of offspring exhibiting both the trait (males) and the preference (females); therefore it is a self-reinforcing system (Fisher 1930, reviewed in Andersson 1994). Sensory drive models of sexual selection postulate that female preferences for certain male traits occur because males are exploiting pre-existing female sensory biases, and emphasize the idea that female preferences may be non-adaptive (reviewed in Endler and Basolo 1998, Ryan 1998). By contrast, the

handicap principle states that only high quality males have the ability to produce the most elaborate traits; therefore, the traits themselves are honest indicators of a male's fitness and females may benefit by preferentially choosing to mate with these males (Zahavi 1975, 1977, Kodric-Brown and Brown 1984, reviewed in Andersson 1994). Females may benefit either indirectly, by obtaining better quality genes for their offspring which may influence offspring survival and reproductive success, or they may benefit directly, via access to higher quality territories or, in species with paternal care, higher male investment into offspring care (reviewed in Andersson 1994). Although different models of sexual selection have been proposed, they are not mutually exclusive; all three may drive the evolution of exaggerated traits, as long as offspring produced from these female preferences have higher reproductive success (Kokko et al. 2002, Andersson and Simmons 2006).

Support for honest indicator theory has been found across taxa, with traits ranging from call duration in male gray tree frogs (*Hyla versicolor*, Welch et al. 1998) to nuptial colouration in three-spined stickleback (*Gasterosteus aculeatus*, Candolin 2000). Honest indicators may reveal information about the male's genetic or phenotypic quality, such as heritable parasite resistance (Hamilton and Zuk 1982), parental effort (Hoelzer 1989), immunocompetence (Folstad and Karter 1992, Wedekind and Folstad 1994), or other aspects of phenotypic quality such as body condition. Generally, the highest quality animals are able to produce the largest, most colourful, and most elaborate traits.

While honest indicator models of sexual selection have been extensively investigated in the context of how male traits are influenced by various measures of

condition, much less attention has been given to the evolutionary forces which drive the elaboration of female traits in some species.

Female ornamentation and mutual sexual selection

Bateman's early work on fruit flies led him to infer that differences in reproductive success between males and females derive from anisogamy, that is, differential investment into gametes by each sex (Bateman 1948). He predicted that this pattern should drive male competition over access to females, since male reproductive success is reliant on his number of mates (Bateman 1948). Trivers then expanded on Bateman's suggestions about parental investment by each sex, suggesting that in species where both males and females invest greatly in offspring, there is the potential for both sexes to be choosy (Trivers 1972). However, females often provide a greater proportion of offspring care, and are therefore usually the choosier sex, driving male-male competition (Trivers 1972). Furthermore, the tendency for females to exhibit higher parental investment has resulted in natural selection driving female crypsis to reduce predation while in proximity to offspring (Darwin 1871, Wallace 1891). These factors have led to the evolution of widespread male showiness and female crypsis. Although many species fit this pattern, there are several species in which both males and females have evolved elaborate traits.

A number of hypotheses have been proposed to explain the evolution of elaborate traits in females (reviewed in Amundsen 2000, Amundsen and Pärn 2006). Firstly, the evolution of a particular trait in males may also lead to the evolution of that trait in females if it has a genetic basis and those genes are shared between the sexes (Lande

1980). The majority of the male and female genome is shared; therefore, when females prefer a certain male trait and drive its evolution, they may then also be driving the development of that trait in females. This is known as the correlated response hypothesis (Lande 1980), and has been proposed as the mechanism driving the evolution of female traits in some species (e.g., Muma and Weatherhead 1989, Hill 1993). If genetic correlation between the sexes leads to expression of both the male trait in females and the female preference for ornamentation in males, then males may exhibit choice for more ornamented females, even when the female trait is non-functional (Hill 1993). Alternatively, there may be genetic correlation between the condition-dependence of the mutual trait, such that females bearing the trait advertise the same quality information as males (Bonduriansky and Rowe 2005). In this case, males who also inherit a preference for showy females may gain either direct (e.g. more maternal care, better nest defence) or indirect (higher quality genes for offspring) benefits. Furthermore, if males have preferences for showy females, then offspring of both sexes may inherit the showiness and boost their reproductive success, enforcing the functionality of the trait in both sexes (Amundsen 2000, Amundsen and Pärn 2006). However, ultimately, the correlated response hypothesis predicts that the same evolutionary pressures which drive the elaboration and maintenance of the trait in males may not necessarily be operating in females, which may result in the expression of non-functional female traits.

Mutual sexual selection theory predicts that both male and female traits may function in intrasexual competition and mate attraction (Parker 1983, Andersson 1994). Female traits may be used in direct competition with other females during the breeding season for access to mates, as with male-male competition. Female traits may also be

under social selection during the non-breeding season, used in competition with either sex over non-sexual resources such as high-quality foraging territories (West-Eberhard 1979, 1983). Male mate choice may also greatly influence the reproductive success of females, since when both males and females pair with preferred mates, they produce higher quality offspring than when males and females pair with non-preferred mates (Drickamer 2003).

Though female choosiness is known to play a large role in pair formation (or simply choice of mates) in many species, male choosiness is rare. Parker (1983) was the first to model the circumstances under which we might predict mutual mate choice to occur; however, his model was based on the unrealistic assumptions that all potential mates are available without temporal restriction, and that there is no search cost to matings. When the component of mate searching behaviour was added to models of mutual mate choice, the importance of determining the amount of information available to individuals when evaluating mates became apparent (Real 1991). Both models also predicted that mutual mate choice should lead to assortative mating (Parker 1983, Real 1991). Assortative mating refers to the non-random pairing of individuals by phenotype or quality (Burley 1983), which may imply mutual preferences for a trait in both sexes. It is important to note that choosiness may also change with timing in the breeding season, such that the highest quality individuals pair up early in the season, with a decrease in discrimination over time (Johnstone 1997).

Logically, it follows to ask under what circumstances the evolution of male choosiness and female ornamentation is favourable. There has been some debate over which factors most greatly influence the evolution of mutual mate choice. While some

argue that the operational sex ratio of the population drives choosiness (e.g. Kvarnemo and Ahnesjö 1996, Parker and Simmons 1996), more recent models of mutual mate choice have revealed that other factors may be of greater importance. For example, the direction of sexual selection should also be influenced by variation in potential mate quality, sex-specific parental investment, species-specific mate encounter rate, sex-specific mate encounter rate, and cost of mate searching (Kokko and Monaghan 2001, Kokko and Johnstone 2002). When only one sex (usually female) provides offspring care, males are free to engage in other breeding attempts, whereas females are limited in their re-mating time by caring for offspring (Johnstone et al. 1996). Therefore, in species where males and females invest equally into offspring care, both suffer the cost of increased mate search time since they cannot re-mate until their offspring have reached independence, therefore they should be choosier about who to mate with in the first place. Offspring survival is also higher when both sexes invest in care, which suggests that the evolution of mutual mate choice is expected to occur in species exhibiting high offspring investment by both males and females (Kokko and Johnstone 2002).

Another area of interest is female signal function, and how it influences the evolution of male choice. In monogamous mating systems, where both males and females often exhibit parental care, theory predicts that both sexes should be choosy (Kokko and Johnstone 2002). Furthermore, mutual mate choice is predicted to occur in species in which biparental care is necessary to ensure offspring survival (Kokko and Johnstone 2002). Even in polygynous mating systems, where males often contribute little or nothing to parental care, male mate choice is favoured when female traits honestly confer information about fertility or viability, and not when they are arbitrary (Servedio and

Lande 2006). However, if the genes controlling both trait expression and preference are correlated between the sexes, then a male preference for arbitrary traits may evolve, resulting in mutual mate choice (Servedio and Lande 2006). Therefore, mutual mate choice is not only expected when female traits are adaptive, but also when there is simply a genetic correlation in both trait and preference between the sexes. When both mates exert preferences for traits which honestly advertise quality, their offspring achieve higher survival rates and future reproductive success owing to enhanced attractiveness (Hooper and Miller 2008). One postulated drawback to female ornamentation is the trade-off between the expression of a female trait and her fecundity, such that a female who invests more into producing an elaborate trait may not be able to invest as many resources into the production of high-quality offspring (Fitzpatrick et al. 2005). However, male preference can exert adaptive, non-directional selection on female traits, driving the maintenance of honest female traits despite this female trade-off (Chenoweth et al. 2006).

The theory of mutual mate choice has been empirically tested in several species to date, and studies have documented male preferences for female traits in birds (reviewed in Amundsen 2000), mammals (e.g. Szykman et al. 2001, Craig et al. 2002), arthropods (Cross et al. 2007, Aquiloni and Gherardi 2008), insects (reviewed in Bonduriansky 2001), and fish (e.g. Kraak and Bakker 1998, Amundsen and Forsgren 2001). However, males do not always exhibit preferences for more ornamented females (eg. Muma and Weatherhead 1989, Murphy 2008), making this an important avenue for future research.

Plumage colouration: mechanisms and condition-dependence

As a taxon, birds are widely known for their elaborate songs and visual displays. As visual ornaments, birds may evolve altered lengths of feathers, overall shapes of feathers, and may also develop extravagantly-coloured feathers. There are two main mechanisms of plumage colouration: pigment deposition into feather keratin (reviewed in McGraw 2006a, b), and the interaction of light with the arrangement of nanostructures within feathers (reviewed in Prum 2006). Pigment-based plumage colouration is generally further subdivided into two major pigment types, although other types exist: carotenoids and melanins.

Carotenoid-based plumage colouration has received a great deal of attention in the study of plumage colouration. Carotenoids are organic pigments produced by plants, which animals may absorb through their direct consumption of vegetation or prey which consumes plants. Many different types of animals metabolize carotenoids to produce colouration ranging from yellow to red, which may be exhibited in feathers, bills, or skin (McGraw 2006a). Since animals cannot synthesize carotenoids for pigmentation, their degree of carotenoid-based colouration relies on environmental carotenoid availability and the animal's own ability to obtain the necessary carotenoids in their diet (Hill 2006). Therefore, the condition-dependence of carotenoid-based plumage colouration has been well-established; those individuals able to obtain and sequester more carotenoids than their counterparts produce more pigmented feathers, and these individuals tend to be of higher quality. Carotenoid-based signals may honestly advertise competitive ability (Pryke et al. 2002, Pryke and Andersson 2003, but see McGraw and Hill 2000a) and quality (e.g. swimming performance, Nicoletto 1991; nutritional condition, Hill and Montgomerie 1994), and males with the most elaborate carotenoid ornaments are often

preferred by females (e.g. Hill 1990, 1991); hence, carotenoid ornamentation is widely accepted as a sexually-selected trait that often functions as an honest signal.

Melanin pigmentation differs from carotenoid pigmentation in that animals are able to synthesize their own melanins via cells in the epidermis or hair follicles named melanocytes (Duval et al. 2002). There are two types of melanins: phaeomelanins and eumelanins, and different mixtures of the two types are responsible for producing the range of melanin-based colours observed (reviewed in Jawor and Breitwisch 2003). While eumelanin-based traits are dark brown to black, phaeomelanins produce colours from rusty brown to red (reviewed in Jawor and Breitwisch 2003). Though melanin pigmentation has been shown to contribute significantly to dominance signalling (reviewed in Senar 1999, Jawor and Breitwisch 2003), and darker, larger patches of melanin-based plumage are preferred by females (e.g. Møller 1998. Sætre et al. 1994), many studies have failed to find a link between melanin colouration and quality (e.g. McGraw and Hill 2000b, McGraw et al. 2002, but see Jawor and Breitwisch 2003, Griffith et al. 2006). In 2008, Roulin and colleagues provided the first direct support for the condition-dependence of phaeomelanin-based ornaments; birds under more stress (as measured by corticosterone level) were not able to deposit as many melanin pigments into their feathers.

Structural feather colouration results from the interaction of light with different refractive indices, and the resulting coherent scattering of light (Prum 2006). The components involved in feathers are typically melanin, air, and keratin; however many other substances may also produce structural colouration. When the nanostructures are laminar and crystal-like, the resulting plumage colouration appears iridescent, where the

colour changes based on viewing geometry. By contrast, quasi-ordered nanostructures produce non-iridescent structural colouration (reviewed in Prum 2006). Non-iridescent structural colouration reflects light in the UV to green range, while iridescent colours may reflect light in any part of the bird-visible spectrum (Prum 2006). Structural colouration is also important in the enhancement of pigment-based colouration (Shawkey and Hill 2005). Studies to date have shown correlations between noniridescent male structural colouration and paternal investment (Keyser and Hill 2000, Siefferman and Hill 2003), body size (Keyser and Hill 2000), and offspring condition (Siefferman and Hill 2003), as well as between iridescent male structural colouration and body size, endoparasite load, and feather growth rate (Doucet 2002, Doucet and Montgomerie 2003). A study on the effect of nutritional condition on structural colour expression demonstrated that brown-headed cowbirds (*Molothrus ater*) fed a restricted diet produced less colourful iridescent plumage colouration than birds that were not nutritionally stressed (McGraw et al. 2002). In another study, endoparasite load also negatively affected the production of iridescent structural colouration when birds were experimentally infected with coccidian parasites (Hill et al. 2005). Variation in the thickness or number of nanostructures within feathers affects colouration (Shawkey et al. 2003, Doucet et al. 2006, Prum 2006), but studies have yet to determine a specific mechanism by which condition alters structural colouration.

The use of reflectance spectrometry to objectively quantify colour

Until recently, the study of biological colour was limited to human-based rankings, or comparisons between plants and animals and colour standards. In the past

decade, scientists interested in objectively quantifying colouration have begun to use reflectance spectrometers, which render the measurement of colour accurate, repeatable, and comparable between studies (reviewed in Andersson and Prager 2006). Reflectance spectrometers employ a bifurcated fiber-optic cable to both direct light onto a specimen and collect reflected light from the specimen (Andersson and Prager 2006). Software enables the visualization of the reflected light via a reflectance spectrograph, which displays wavelength on the x-axis, and percent reflectance on the y-axis (Andersson and Prager 2006). From these spectrographs, researchers can calculate a number of different variables, including brightness, saturation, and hue, which represent the three main dimensions of colour (Hailman 1979, Endler 1990, Montgomerie 2006). Avian biologists evaluating feather colouration usually focus on the portion of the spectrum between 300 nanometers (ultraviolet light) and 700 nanometers (red light), because most birds, and indeed all passerines studied to date, are able to see colours within this range of wavelengths (Cuthill 2006).

Study species

The royal flycatcher (*Onychorhynchus coronatus*) is a small neotropical passerine belonging to the family Tyrannidae (Fitzpatrick 2004). This family is notable for its size, comprising roughly 400 species in over 100 genera (Ohlson et al 2008). Most members of this family exhibit drab plumage, though many species develop colourful crest feathers which are usually concealed but can be exposed during social interactions. Royal flycatcher crest feathers exhibit remarkably exaggerated examples of this feature in colour, patterning and length; both male and female royal flycatchers produce large,

elaborately coloured fan-shaped crests (Skutch 1960). Male royal flycatcher crests are mostly red in colour, with black spots, and blue iridescent tips. Female crests are mostly yellow, with black spots and iridescent tips. Royal flycatchers selectively display their crests; the majority of the time, crests are folded and concealed, giving them their characteristic "hammerhead" appearance (Skutch 1960). Both sexes also display colourful pale yellow rump plumage, which is their most striking plumage feature when crests are concealed.

While these birds have fascinated researchers for nearly a century, there have been no comprehensive studies of their behaviour or ecology. In 1910, Peck commented on their curious nest structures, among those of other species. Royal flycatchers build long, pendulous nests which appear to mimic post-flood debris and present a logistic challenge to would-be predators due to their location high above rivers (Peck 1910, Skutch 1960). In 1960, acclaimed ornithologist Alexander Skutch published a longer account of their natural history, where he provided the first description of royal flycatcher crests and of their breeding behaviour (Skutch 1960), although these observations were limited to a few unmarked individuals. Skutch (1960) observed that birds display their crests while preening, and witnessed males displaying to females. He also reported that collectors affirmed the function of these crests in conspecific fights, though Skutch himself never witnessed the crest display during an antagonistic interaction (Skutch 1960). More recently, Graves (1990) published a compilation of possible hypotheses for royal flycatcher displays and an anecdotal observation of two birds displaying to each other (Graves, 1990). Finally, in 2006, Chaves published an observation of two birds copulating, during which both the male and the female erected their crests in full display

(Chaves, 2006). In all, most published accounts of royal flycatchers to date have centered on reporting brief encounters with these birds, and none have sought to provide either strong empirical or experimental data to explain the evolution and function of the elaborate crest displays of male and female royal flycatchers. However, these anecdotal observations suggest that the crests are used during courtship, intrasexual aggression, and even interspecific interactions.

Honest advertisement and mutual mate choice in the royal flycatcher

Both male and female royal flycatchers display elaborate plumage colouration. The overall goal of my thesis was to investigate whether or not plumage colouration functions as a sexually selected trait in both males and females. In Chapter 2, I determined whether royal flycatcher plumage colouration revealed quality to investigate whether honest indicator models of sexual selection could be driving the evolution of showy male and female traits in this species. If both male and female traits reveal quality, individuals of both sexes could benefit by pairing with an elaborately coloured individual of the opposite sex. I measured plumage colour variation in males and females, including juvenile males who we discovered exhibit delayed plumage maturation. I then quantified several quality indicators for both males and females, and compared these to plumage colouration. In Chapter 3, I investigated mutual mate choice by determining whether or not royal flycatcher plumage colouration predicted reproductive success, and whether individuals mated assortatively by plumage colouration, morphology, or quality. These studies provide the first in-depth qualitative and quantitative investigations of the function of plumage colouration in royal flycatchers, and suggest many future avenues

for research in this dynamic species. Both data chapters were written in preparation for submission to journals: Chapter 2 is currently in review at Animal Behaviour, and we are preparing Chapter 3 for submission to The Auk.

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CHAPTER 2: ELABORATE ORNAMENTS IN NEOTROPICAL ROYAL FLYCATCHERS: DOES PLUMAGE SIGNAL QUALITY IN BOTH SEXES?

Synopsis

Traditionally, studies of sexual selection have focused on showy males displaying to choosy females. In many cases, elaborate ornaments appear to signal some aspect of male quality to potential mates. Females of many species also express some degree of ornamentation, and although female ornaments may evolve through correlated evolution by selection on males, recent studies suggest that, in some species, selection may also directly favour female ornamentation. Royal flycatchers (*Onychorhynchus coronatus*) exhibit pronounced mutual ornamentation: both males and females display elaborate crests which exhibit phaeomelanin-based and iridescent colouration, and both sexes exhibit pale yellow rump feathers and rufous rectrices. In this study, we sought to characterize sexual dimorphism and dichromatism in royal flycatchers, and to investigate the possibility that plumage colouration might honestly reveal quality in both males and females. We used reflectance spectrometry to measure the colouration of crest, rump, and rectrix feathers, and analyzed whether plumage colouration correlated with immunocompetence, condition, and ectoparasite load. Additionally, we investigated whether female colouration honestly advertised maternal quality by assessing nestling provisioning rate. We documented substantial sexual dimorphism and dichromatism in royal flycatchers, and delayed plumage maturation in males. We also uncovered associations between plumage colouration and quality indices in both males and females, including female nestling provisioning rates, particularly in relation to phaemelanin-based colouration. Our study is among the first to directly compare male and female traits with several measures of quality in the investigation of honest signals, and contributes to our understanding of the evolution of elaborate traits in both sexes.

Introduction

Sexual selection acts on individuals through mate choice and competition with rivals, often driving the evolution of elaborate traits, particularly in males (Andersson 1994a). Although elaborate male ornaments may evolve through a variety of mechanisms, one way to explain female preference for showy male traits is through the good genes model, which states that females base reproductive decisions on their evaluation of males' secondary sexual traits, and that these traits may confer information about male quality which will ultimately benefit her offspring (Andersson 1994a). The honest advertisement hypothesis assumes that these elaborate traits are costly to produce and maintain, thus enforcing their sexual signal honesty (Zahavi 1975, 1977, Kodric-Brown and Brown 1984).

Honest advertisement studies have traditionally focused on identifying the relationship between male signals and various measures of quality. In particular, many studies have investigated the link between plumage colour and quality, and there is wide support for male visual signals as indicators of quality (reviewed in Hill 2006). Despite the overwhelming prevalence of male showiness across taxa, there are many species in which females are ornamented to varying degrees (Amundsen 2000). Some females display a less elaborate version of male ornaments (e.g., Hill 1993, Amundsen et al. 1997), some females match their males in showiness (e.g., Jones and Hunter 1993, Murphy 2007), and some surpass them (Heinsohn and Legge 2003). Female ornamentation is generally thought to evolve through one of two primary mechanisms (Amundsen and Pärn 2006). The correlated response hypothesis postulates that females

express ornaments as a by-product of their selection on male genes for showy traits (Lande 1980). By contrast, female ornamentation can be maintained through female-female competition or male preference for elaborate female traits (Darwin 1871, Amundsen 2000, Amundsen and Pärn 2006). Social selection may occur when female traits are advantageous not for mate attraction, but rather because they confer an advantage during interactions in nonsexual contexts such as territory defence (West-Eberhard 1979). When both males and females preferentially mate with highly ornamented individuals, mutual sexual selection may drive the elaboration of traits in both sexes (Andersson 1994a, Johnstone et al. 1996). For example, both male pipefish (*Nerophis ophidion*) and two-spotted gobies (*Gobiusculus flavescens*) prefer to mate with colourful females (Berglund et al. 1986, Amundsen and Forsgren 2001). Despite the historic bias for studying sexual selection exclusively in the context of how male ornaments and female preferences affect reproductive success, it is also important to acknowledge the role of male choice in male reproductive success. By preferentially mating with a high quality female, males may gain a variety of direct and indirect benefits, including better genes, or increased maternal care for their offspring (good parent process, Hoelzer 1989).

Though examples of female signals of quality exist across taxa (e.g. Berglund et al. 1997, Domb and Pagel 2001), the relatively few studies conducted to date have found mixed support for the hypothesis that avian female ornamentation signals quality. For example, in female red-winged blackbirds (*Agelaius phoeniceus*), epaulet colouration correlates with her condition in the previous year (Johnsen et al. 1996), and in pied flycatchers (*Ficedula hypoleuca*), the presence of a white forehead patch in females was

negatively correlated with trypanosome infection (Potti and Merino 1996). By contrast, some studies have found no relationship between female ornamentation and nestling feeding rate in bluethroats (*Luscinia svecica*, Rohde et al. 1999), or between female tail length and reproductive success in turquoise-browed motmots (*Eumomota superciliosa*, Murphy 2007). Species exhibiting mutual ornamentation (where both sexes exhibit the same sort of ornamentation) provide the opportunity to perform a direct comparison between male and female quality and the expression of a trait. For instance, a study of yellow-breasted chats (*Icteria virens*, Cooper and Ritchison 2005) found no correlation between male and female morphology (including colouration) and nestling provisioning. Our goal in this study was to investigate the relationship between plumage ornamentation and individual quality in male and female royal flycatchers (*Onychorhynchus coronatus*).

Royal flycatchers are Neotropical passerines belonging to the family Tyrannidae, a speciose group of New World flycatchers (Fitzpatrick 2004). While most members of Tyrannidae are relatively cryptic in colouration and sexually monomorphic, royal flycatchers have elaborate fan-shaped crests that are red in males and yellow in females, with black spots and iridescent blue tips in both sexes (Fig. 2.1). These crests may be concealed or displayed at will, and appear to play an important role during courtship and copulation (Graves 1990, Chaves 2006). The erection of crest feathers is often accompanied by mesmerizing snake-like movements of the head and also by display flights. Despite the compelling nature of royal flycatcher visual signals, this species remains virtually unstudied. Our observations of colour-marked individuals revealed that these birds are facultatively polygynous, as some males regularly paired with two nesting females. In this study, we characterize sexual dimorphism and dichromatism in royal

flycatchers, and investigate the relationship between plumage colouration and measures of quality in both sexes.

Methods

From 2003 to 2007, we studied free-living royal flycatchers in Santa Rosa National Park, Area de Conservacíon Guanacaste, Costa Rica. Most data were collected during the 2006 and 2007 breeding seasons, however a few birds included in our analyses were caught between 2003 and 2005. At our study site, royal flycatchers begin nest-building in April, before the onset of the rainy season, and breed until July or August. Royal flycatchers build long, pendulous nests high over rivers and roads (Peck 1910, Skutch 1960). We captured these birds by placing mist nets near active nests, and assigned each individual a unique combination of metal or plastic coloured leg bands for later re-identification. During capture, we recorded standard morphological measurements for each individual. In particular, we measured the tarsus length, mass, culmen length, wing chord, and tail length of every adult captured. We measured maximum crest height (from the base of the central crest feathers to the tip of the central crest feathers when the crest was fully erect) as a proxy for crest size. We also collected feathers for ptilochronological and spectral analyses, and a collected a small blood sample to prepare blood smears. We monitored nests throughout the breeding period and recorded the identity of the territory owners. Some of the males we captured had orange, rather than red, crests. We assume that these were juvenile (second year) males because they had shorter wings (Table 1) and weighed less than adult males, and never held

breeding territories. Because of these differences in colour, morphology, and behaviour, we considered them separately in our analyses.

Since the mechanisms for plumage colour production in the royal flycatcher are currently undocumented, we performed a simple phaeomelanin extraction procedure (McGraw 2006) and determined that phaeomelanins are at least partly responsible for royal flycatcher crest feather colouration. Adult male royal flycatcher crest feathers leached more phaeomelanins during the extraction process than did female crest feathers, suggesting that sex differences in crest colouration may be due in part to increased phaeomelanin deposition by males.

Reflectance spectrometry

With the exception of the colourful crest, royal flycatcher body plumage is mostly olive-brown above and tawny yellow below, with both males and females exhibiting a bright yellowish-buff rump patch and rufous tail (Skutch 1960). We collected at least five feathers from each of the crest, and rump, as well as the right outer rectrix from each adult in our population. In the lab, we overlapped the feathers from each body region to approximate their placement on a bird and affixed them to pieces of black cardboard. To objectively quantify colour, we measured plumage reflectance with a USB4000 spectrometer (Ocean Optics, detector range 200-850 nm) connected to a Deuterium Tungsten Halogen light source (DH2000, Ocean Optics). We used a fiber-optic probe to deliver light to our specimens and collect reflected light from these specimens. A black rubber sheath, angled at 90°to our measurement surface, maintained the probe at a fixed distance from our sample and excluded all external light. All measurements were

collected relative to a Spectralon diffuse white standard (Labsphere, >97% reflectance). We collected five measurements per body region (including both the iridescent and non-iridescent part of the crest), each of which comprised 20 readings averaged by the spectrometer operating software (OOIBase 32). We used averages of these five measurements in our analyses.

When analyzing our reflectance data, we focused exclusively on the portion of the electromagnetic spectrum to which most avian visual systems are sensitive (300-700 nm; reviewed in Cuthill 2006). To summarize our reflectance data, we focused on the three main dimensions of colour, namely brightness, saturation and hue (Endler 1990, reviewed in Montgomerie 2006), using CLR Color Analysis Programs to calculate these variables (Montgomerie 2008). For all body regions, we calculated brightness as the mean brightness from 300 to 700 nm and saturation as the maximum minus minimum reflectance divided by the mean brightness. With the exception of the iridescent portion of the crest, royal flycatcher reflectance spectra had no distinct peaks, and rather increased steadily with wavelength (Fig. 2.2). We therefore used the wavelength at the reflectance midpoint as our measure of hue for these regions, whereas we calculated hue as the wavelength of maximum reflectance within the UV part of the spectrum (300-400 nm) for the iridescent crest.

Condition

We evaluated feather growth rate as a measure of nutritional condition. Faint bars appear on feathers in accordance with daily growth cycles, with light bars representing nocturnal growth and dark bars representing diurnal growth (Grubb 2006). By counting

pairs of light and dark bars in a given section of the feather, and by measuring the length of the section examined, we can estimate daily feather growth rate. Experimental studies have shown that feather growth rate can indicate a bird's nutritional condition at the time it moulted its feathers (reviewed in Grubb 2006). We calculated feather growth rate from the right rectrix feather of each adult in our population. Finally, we examined the subcutaneous fat deposit in the furculum of each adult bird and assigned them a fat score on a scale of 0 to 5 (0 when no fat present, and 5 for bulging fat; see Wingfield and Farner 1978). Although fat deposits can vary diurnally (reviewed in Bednekoff and Houston 1994), our observations suggest that both total and diurnal variation are less pronounced in tropical birds. We also attempted to control for diurnal variation by measuring all birds at roughly the same time of day (between 6 am and 10 am) to minimize these effects.

Parasite load

To evaluate a bird's ectoparasite load, we separately estimated the number of mites on the crest and wing feathers of each individual, which are easily detected by the naked eye during capture when feathers are spread out (see Clayton and Walther 1997). Originally, we sought to evaluate blood parasite levels as another measure of quality, however upon examination of blood smears prepared in the field, we determined that our population of royal flycatchers lacked blood parasites, and we were unable to include this measure in our analysis.

Immunocompetence

To estimate royal flycatcher immunocompetence, we examined heterophil to lymphocyte ratio (H/L) for each individual in our population. Heterophils are granular leukocytes with lobed nuclei which exhibit phagocytic activity (Campbell and Ellis 2007). Though it has been suggested that heterophil to lymphocyte ratio may not always accurately reflect a bird's condition, many studies have shown that heterophil to lymphocyte ratio is a good measure of immune stress (reviewed in Maxwell 1993), and this measure has been used to assess immunocompetence in a wide array of birds (e.g. Moreno et al. 2002, Campo et al. 2008). We prepared blood smears in the field from fresh blood samples taken from each adult royal flycatcher. These smears were later fixed and stained in the lab (Hema III, Fisher Scientific). We used a Zeiss Axiostar Plus microscope to count the number of heterophils, lymphocytes, and other white blood cells per 10,000 red blood cells. In instances where no heterophils or lymphocytes were encountered within 10,000 red blood cells, we continued searching fields of view until at least one of each category was found, and then standardized the values. To normalize the data, we log transformed the heterophil to lymphocyte ratios used in our analyses.

Female quality

In royal flycatchers, females are solely responsible for parental care (Skutch 1960, Cuthbert and Doucet, unpubl. data). We calculated female nestling feeding rates based on nest observations collected during the breeding season. Observations typically lasted two hours, and were conducted throughout the nestling stage. We collected most of our observations during the early nestling period and found no relationship between feeding rate and either nestling age (r = -0.16, N = 34, P = 0.35) or number of nestlings

(ANOVA, $F_{1,32} = 1.57$, $P = 0.22$), and we therefore simply calculated the average rate for each female.

Statistical analyses

To evaluate sexual dimorphism and dichromatism in royal flycatchers, we used Analyses of Variance (ANOVAs) followed by Tukey-Kramer tests to determine significant differences between groups. To analyze the relationships between plumage colour and individual quality, we constructed backward stepwise regressions using five quality variables as dependent variables (feather growth rate, heterophil to lymphocyte ratio, crest parasite load, wing parasite load, and fat score) and eight plumage colour traits as possible predictors (brightness and saturation for each of the iridescent part of the crest, the pigmented part of the crest, the rectrix, and the rump). We did not include measurements of hue in these analyses because hue and saturation were correlated for all body regions ($P < 0.05$). With pigment-based colouration, unless changes in pigment type lead to variation in feather colouration, we predict that changes in pigment concentration are most reflected by changes in brightness and hue (Saks et al. 2003). We set the probability to enter and leave the model at 0.25 and 0.06, respectively.

Results

Dimorphism

Adult male, juvenile male, and female royal flycatchers exhibited significant differences in body size for all morphological traits we measured (Table 2.1). In particular, there was strong male-biased sexual size dimorphism for tarsus length, tail

length, and culmen length, with both adult and juvenile males being significantly larger than females, but not significantly different from each other, for these traits (Table 2.1). For wing chord and body mass, we found evidence of both sexual size dimorphism and delayed maturation, with significant differences between all three sex/age categories, and juvenile males exhibiting an intermediate size between larger adult males and smaller females (Table 2.1).

Dichromatism

We found significant differences among female, adult male, and juvenile male royal flycatchers for most plumage traits we measured (Table 2.2). In particular, female crests were shorter than those of adult males, with juvenile males exhibiting crests of intermediate length. We also documented extensive sexual dichromatism (Fig. 2.2), and evidence of delayed plumage maturation in male royal flycatchers. For pigmented body regions, including the rump, rectrix, and pigmented part of the crest, adult males generally exhibited lower brightness, higher saturation, and longer wavelength hues than females, with juvenile males exhibiting intermediate patterns of reflectance (Table 2.2). Two exceptions to this pattern were the brightness of the rump, and the saturation of the rectrix, which did not differ between the three groups. There were also significant differences for the iridescent part of the crest, with adult males being brighter and more saturated than females and juvenile males; the three groups did not differ in hue for this trait (Table 2.2).

Quality indicators

We used stepwise multiple regression models with backward elimination to examine whether plumage colouration can predict variation in multiple quality indicators in royal flycatchers. In each model, a quality indicator served as the dependent variable, and colourimetric traits served as possible predictor variables. We used separate models for adult males, juvenile males, and females.

In adult males, feather growth rate was positively predicted by the brightness of red crest feathers (Table 2.3). Crest parasite load was positively predicted by the brightness of the red crest and rectrix feathers (Table 2.3). Heterophil to lymphocyte ratio was positively predicted by the saturation of iridescent crest feathers, and negatively predicted by the saturation of the red crest feathers (Table 2.3). Wing parasite score and fat score were not predicted by any of our colourimetric variables for adult males (Table 2.3).

In juvenile male royal flycatchers, feather growth rate was negatively predicted by the saturation of the iridescent crest (Table 2.4). Fat score was positively predicted by the saturation of the orange crest (Table 2.4). Plumage colouration did not significantly predict variation in heterophil to lymphocyte ratio, wing parasite load, or crest parasite load (Table 2.4).

In female royal flycatchers, heterophil to lymphocyte ratio was positively predicted by the brightness of the rump, and negatively predicted by the saturation of the rectrix (Table 2.5). Fat score was negatively predicted by the saturation of the yellow crest (Table 2.5). Nestling feeding rate was positively predicted by the brightness of the yellow crest and rump, and negatively predicted by the brightness of iridescent crest tips and rectrix feathers and the saturation of rectrix feathers (Table 2.5). No female plumage

colour variables were significant predictors of feather growth rate, wing parasite score, or crest parasite load in female royal flycatchers (Table 2.5).

As a proxy for crest size, we also evaluated whether crest height might serve as an indicator of quality independent of crest colour. There was a significant negative relationship between adult male crest height and crest parasite load (Pearson correlation, $r = -0.7$, $n = 25$, $P = 0.0001$) such that males with the longest crest feathers had the fewest crest parasites. Crest height was not significantly correlated with any other measures of quality in adult male, female, or juvenile male royal flycatchers (all $P > 0.10$).

Discussion

To date, most studies of sexual selection have focused on investigating male ornamentation in temperate species. Here, we investigated the possible signal function of elaborate plumage traits in male and female Neotropical royal flycatchers. We discovered that royal flycatchers are strongly sexually dimorphic, both in morphology and colour. Adult males were significantly larger than adult females for all morphological traits we measured. Sexual size dimorphism is commonly attributed to either sexual selection (Andersson 1994a), or, less commonly, to differing ecological pressures between the sexes (Shine 1989). Males are often the larger sex due to size advantages during competition with rival males for access to resources such as females and high quality territories (Andersson 1994a), and polygynous species usually exhibit the most pronounced male-biased sexual size dimorphism (Dunn et al. 2001). Despite the elaboration of female plumage in royal flycatchers, traditional male-biased sexual size dimorphism is still present in this facultatively polygynous species. Polygyny may occur

when there is a male-biased operational sex ratio, which leads to more intense male-male competition for access to mates (Emlen and Oring 1977, Kvarnemo and Ahnesjö 1996). Intrasexual male competition may be further increased by the choosiness of females, who are the sole providers of nestling care in royal flycatchers. Pronounced sexual size dimorphism in royal flycatchers is likely promoted by both of these factors, as has been found in studies across avian families (Payne 1984) and particularly in New World blackbirds (Icterinae, Webster 1992). Interpreting the evolutionary basis for sexual size dimorphism in royal flycatchers will require a better understanding of the pressures of intrasexual competition in both sexes.

Our analyses also revealed extensive sexual dichromatism in the royal flycatcher. Some sex differences in crest colouration were already apparent to the naked eye, particularly the red, orange, and yellow crests of adult males, juvenile males, and females, respectively. However, reflectance spectrometry allowed us to document sex differences in the colouration of the iridescent part of the crest and other body regions which we could not detect visually. In particular, the yellow rumps, buffy rectrices, and red crest feathers of adult males were generally darker (less bright) and more saturated than those of females. Males also had brighter and more saturated UV/blue iridescent crest feather tips. Proximately, these differences in plumage colouration are likely due to differences in colour production mechanisms between the sexes. For pigmented regions, our extraction analysis revealed that male feathers appear to contain more phaeomelanin pigments than female feathers. Since pigments absorb light, an increase in pigment concentration is consistent with the increased saturation and decreased brightness we documented in male plumage patches. Sex differences in the shape of reflectance spectra

also suggest that different types of melanin may be involved in shaping sex differences in colour. Sex differences in pigment colours may also be affected by the underlying feather nanostructure, as has been shown in carotenoid colouration (Shawkey and Hill 2005). Iridescent colouration is produced by the interaction between light and organized nanostructures in the feathers (Prum 2006). Variation in the dimensions of these nanostructures is known to influence colouration both within (Doucet et al. 2006) and among (Shawkey et al. 2006) species, and is likely responsible for producing the sex differences in iridescence we observed in royal flycatcher crest tips. Since brightness and saturation have been shown to be informative traits in other species with iridescent plumage (e.g., McGraw et al. 2002, Doucet and Montgomerie 2003), it is perhaps not surprising that male royal flycatchers had brighter and more saturated iridescent plumage. Sex differences in rump and rectrix colour are also of interest because although crests are concealed most of the time, there are no temporal limitations on the visibility of rump or rectrix feathers, and these body regions may act as visual signals when crests are concealed.

Aside from revealing pronounced dimorphism in royal flycatchers, our study is the first to report evidence of delayed plumage maturation in this species, with juvenile males displaying morphology and plumage colouration intermediate to that of adult males and females (Selander 1965). In species with long life spans and high competition for limited breeding habitat, males may forgo breeding in their first year, and such delayed breeding is often accompanied by delayed plumage maturation (Studd and Robertson 1985). A number of hypotheses have been proposed to explain why juvenile males might exhibit delayed plumage maturation, including crypsis, female mimicry,

moult constraints or food limitation, and status signalling (reviewed in Owens 2006). It seems unlikely that selection for crypsis would explain delayed plumage maturation in royal flycatchers since their crests are usually folded and concealed. However, the female mimicry, moult constraint, and status signalling hypotheses remain contenders for explaining delayed plumage maturation in this species. Royal flycatchers present an interesting avenue for future tests of hypotheses explaining the evolution of delayed plumage maturation.

One key objective of our study was to investigate the link between plumage colouration and quality in both male and female royal flycatchers. In adult males, crest parasite load was positively predicted by the brightness of the red crest and the rectrix; males with darker, more pigmented feathers had fewer parasites. Males with larger crests also had fewer parasites. These data support parasite-mediated models of sexual selection (Hamilton and Zuk 1982), and suggest that ornamental crests honestly reveal freedom from parasites in royal flycatchers. We also found that the brightness of the red crest was a significant positive predictor of feather growth rate. These findings run contrary to expected patterns, since more pigmented males grew their feathers more slowly, and slower feather growth should indicate poor nutritional condition (Grubb 2006). Such a pattern might be expected, for example, if high quality individuals expend so much energy during the breeding season that they are in poor condition during post-breeding molts (e.g., Andersson 1994b, Doucet and Montgomerie 2003). Alternatively, perhaps in tropical species, which do not face the pressure of an oncoming migration or a looming winter, individuals can afford to grow their feathers more slowly than temperate birds. If this is the case, slower growth might actually enhance pigment deposition, as has recently

been shown for carotenoid-based colouration (Serra et al. 2007). Finally, we found that heterophil to lymphocyte ratio was positively predicted by the saturation of the iridescent crest and negatively predicted by the saturation of the red crest. Since elevated heterophil levels indicate stress (Maxwell 1993), our data suggest that healthier males have less saturated iridescent crests, but more saturated red crests, indicating a possible trade-off between iridescent and red crest colouration.

Ultimately, our findings suggest that male phaeomelanin-based colouration could serve as an honest signal of quality in adult male royal flycatchers. In the extensive body of work on condition-dependent plumage signalling, phaeomelanin colouration has received less attention than carotenoid, eumelanin, or structural plumage colours (Hill 2006), although there is some evidence that it may be a sexually selected trait in both intrasexual (Studd and Robertson 1985) and intersexual contexts (e.g. King et al. 2001, Safran and McGraw 2004). Furthermore, a recent study documented a relationship between corticosterone levels and phaoemelanin plumage colouration, such that birds undergoing more stress develop less pigmented plumage (Roulin et al. 2008). It is interesting to note that our measure of a different stress indicator, heterophil to lymphocyte ratio, was also related to phaeomelanin-based plumage colouration in royal flycatchers. This evidence provides support for condition-dependent signalling of phaeomelanin-based ornaments.

In juvenile male royal flycatchers, feather growth rate was negatively predicted by the saturation of the iridescent crest. As with adult males, these data suggest that individuals growing their feathers more slowly developed more elaborate plumage colouration. Doucet and Montgomerie (2003) documented a similar relationship in

another tropical species with iridescent plumage. Perhaps, as with carotenoid-based plumage (Serra et al. 2007), slower feather growth facilitates the development of nanostructures responsible for creating iridescent plumage in this species. We also found that fat score was positively predicted by the saturation of the orange crest, suggesting that juvenile males with more saturated orange crowns are better able to maintain fat stores. Plumage colouration was also shown to be positively correlated with fat score in blue tits (*Parus caeruleus,* Svensson and Merilä 1996), but not in blue grosbeaks (Keyser and Hill 2000). Overall, though we found fewer quality indicators in juveniles males than in adults, these results suggest that juvenile crest plumage colouration is a condition-dependent signal.

In female royal flycatchers, heterophil to lymphocyte ratio was positively predicted by the brightness of the rump and negatively predicted by the saturation of the rectrix, such that females experiencing higher immune stress had less pigmented rumps and rectrices. Thus, there appears to be a negative relationship between pigmentation and stress in both males and females. However, fat score was negatively predicted by the saturation of the yellow crest, suggesting that females with less saturated crests actually had higher fat reserves. Finally, female nestling provisioning rate was positively predicted by the brightness of the yellow crest and the rump, and negatively predicted by the brightness of the iridescent crest and the brightness and saturation of the rectrix. These data suggest that there might be a trade-off between condition and maternal quality. For example, studies of socially monogamous northern cardinals found that more colourful females had higher nestling provisioning rates (Linville et al. 1998, Jawor et al 2004), but also higher total leukocyte counts, suggesting that more colourful females

experienced higher levels of immune stress, perhaps because they invested so much in parental care (Maney et al. 2008). Previous investigations have generally supported the condition-dependence of female plumage traits (e.g., Velando et al. 2001, Roulin et al. 2001a, Potti and Merino 1996, but see Hill 1993); however, several studies found no relation between female colouration and maternal quality (Rohde et al 1999, Smiseth and Amundsen 2000, Roulin et al. 2001b, Cooper and Ritchison 2005, Siefferman and Hill 2005, Morales et al. 2007). Although male royal flycatchers do not invest in parental care, and might therefore experience low costs to indiscriminate mating, high variance in female quality may provide an incentive for male choosiness (Parker 1983, Johnstone et al. 1996), especially if the population supports more females than can be accommodated by breeding territories. Male royal flycatchers may gain significant direct benefits by pairing with high quality females if they provision nestlings more frequently. Alternatively, females may exhibit quality indicating traits as the result of genetic correlation between the sexes (Bonduriansky and Rowe 2005). However, this is unlikely in royal flycatchers since different male and female traits revealed different quality information, and crests in particular are not similar in colour between the sexes. Future studies should determine whether male choice plays a role in sexual selection in this species.

In conclusion, we documented extensive sexual dichromatism and delayed plumage maturation in royal flycatchers, and found support for honest advertisement models of sexual selection in both males and females, although further study will be required to determine whether royal flycatchers exploit the information content of these plumage signals. Our findings also suggest that multiple plumage traits may reveal

different aspects of quality, and that indices of quality may trade-off against one another. These observations stress the importance of considering multiple traits and multiple measures of quality when investigating honest advertisement in a species, and highlight the need for experimental manipulations of these traits in future studies. Our study makes an important contribution to our limited understanding of the function of mutual ornamentation in birds.

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Table 2.1. Sexual dimorphism in morphological traits in royal flycatchers

Body measurement	Adult male	Juvenile male	Female	*F*	df	*P*
Tarsus length (mm)	17.69[a] ± 0.51	17.55[a] ± 0.52	16.73[b] ± 0.63	24.4	2, 75	<0.0001
Tail length (mm)	68.52[a] ± 2.09	67.62[a] ± 2.40	64.01[b] ± 1.80	42.3	2, 73	<0.0001
Wing chord (mm)	86.96[a] ± 1.39	84.65[b] ± 1.76	79.03[c] ± 2.55	111.8	2, 73	<0.0001
Culmen length (mm)	16.35[a] ± 0.66	16.31[a] ± 0.55	15.20[b] ± 0.58	34.7	2, 76	<0.0001
Mass (g)	21.56[a] ± 0.89	20.13[b] ± 0.61	18.44[c] ± 1.73	41.9	2, 74	<0.0001

All means are reported ± standard deviation. Results from a post-hoc Tukey-Kramer test are indicated with superscript letters, where values with different letters are significantly different from each other ($P < 0.05$). Sample sizes were $N = 26$ (adult males), $N = 13$ (juvenile males), $N = 39$ (females).

Table 2.2. Sexual dimorphism and dichromatism of plumage traits in royal flycatchers

Region	Trait	Adult male	Juvenile male	Female	*F*	df	*P*
Crest	Height (mm)	$29.33^{a} \pm 1.18$	$27.0^{b} \pm 0.68$	$25.35^{c} \pm 1.39$	79.8	2, 73	<0.0001
Rectrix	Brightness (%)	$13.80^{b} \pm 1.10$	$14.82^{a} \pm 0.99$	$14.65^{a} \pm 1.23$	23.9	2, 78	<0.0001
	Saturation	1.19 ± 0.11	1.18 ± 0.11	1.20 ± 0.12	0.1	2, 78	0.89
	Hue (nm)	$541.54^{a} \pm 7.81$	$533.67^{b} \pm 7.00$	$532.38^{b} \pm 10.25$	8.5	2, 78	0.0004
Rump	Brightness (%)	19.38 ± 4.12	20.24 ± 2.88	19.97 ± 4.21	0.3	2, 72	0.78
	Saturation	$2.00^{a} \pm 0.29$	$1.83^{ab} \pm 0.26$	$1.78^{b} \pm 0.27$	5.1	2, 72	0.009
	Hue (nm)	$536.78^{a} \pm 15.14$	$524.74^{b} \pm 16.07$	$517.74^{b} \pm 13.71$	12.7	2, 72	<0.0001
Pigmented crest	Brightness (%)	$12.14^{c} \pm 0.99$	$15.47^{b} \pm 1.56$	$18.79^{a} \pm 2.03$	125.5	2, 78	<0.0001
	Saturation	$3.97^{a} \pm 0.20$	$3.12^{b} \pm 0.22$	$2.28^{c} \pm 0.21$	521.4	2, 78	<0.0001
	Hue (nm)	$616.81^{a} \pm 2.65$	$591.61^{b} \pm 12.08$	$541.91^{c} \pm 9.27$	666.6	2, 78	<0.0001
Iridescent crest	Brightness (%)	$17.81^{a} \pm 2.28$	$15.78^{ab} \pm 3.28$	$15.30^{b} \pm 3.80$	4.8	2, 78	0.01
	Saturation	$0.78^{a} \pm 0.09$	$0.69^{b} \pm 0.09$	$0.71^{b} \pm 0.11$	4.3	2, 78	0.02
	Hue (nm)	336.78 ± 6.74	340.63 ± 5.75	336.50 ± 8.02	1.8	2, 78	0.18

All means are reported ± standard deviation. Results from a post-hoc Tukey-Kramer test are indicated with superscript letters, where values with different letters are significantly different from each other ($P < 0.05$). Sample sizes were $N = 25$ (adult males), $N = 13$ (juvenile males), $N = 37$ (adult females).

Table 2.3. Significant predictors of quality in adult male royal flycatchers using stepwise regressions with a backward elimination procedure.

Dependent variable	Model predictors	R^2 or β'	*F*	Df	*P*
Feather growth rate	Whole model	0.19	5.6	1,23	0.03
	Red crest brightness	0.44	5.6	1,23	0.03
Wing parasite score	No significant predictors				> 0.22
Crest parasite load	Whole model	0.31	5.1	2,22	0.02
	Red crest brightness	0.48	7.1	1,23	0.01
	Rectrix brightness	0.39	4.7	1,23	0.04
Log (H/L)	Whole model	0.32	3.7	2,16	0.05
	Red crest saturation	-0.51	4.6	1,17	0.05
	Iridescent crest saturation	0.60	6.39	1,17	0.02
Fat score	No significant predictors				> 0.22

Table 2.4. Significant predictors of quality in juvenile male royal flycatchers using stepwise regressions with a backward elimination procedure.

Dependent variable	Model predictors	R^2 or β'	F	df	P
Feather growth rate	Whole model	0.32	5.3	1,11	0.04
	Iridescent crest saturation	-0.57	5.3	1,11	0.04
Wing parasite score	No significant predictors				> 0.12
Crest parasite load	No significant predictors				> 0.20
Log (H/L)	No significant predictors				> 0.04
Fat score	Whole model	0.33	5.5	1,11	0.04
	Orange crest saturation	0.58	5.5	1,11	0.04

Table 2.5. Significant predictors of quality in female royal flycatchers using stepwise regressions with a backward elimination procedure.

Dependent variable	Model predictors	R^2 or β'	*F*	df	*P*
Feather growth rate	No significant predictors				> 0.07
Wing parasite score	No significant predictors				> 0.16
Crest parasite load	No significant predictors				> 0.18
Log (H/L)	Whole model	0.35	7.3	2,27	0.003
	Rump brightness	0.41	6.6	1,28	0.02
	Rectrix saturation	-0.32	4.1	1,28	0.05
Fat score	Whole model	0.35	19.3	1,34	< 0.0001
	Yellow crest saturation	-0.59	19.3	1,34	< 0.0001
Nestling feeding rate	Whole model	0.87	10.5	5,8	0.002
	Yellow crest brightness	0.40	8.8	1,12	0.02
	Iridescent crest brightness	-0.34	5.1	1,12	0.05
	Rump brightness	0.46	11.0	1,12	0.01
	Rectrix brightness	-1.12	30.0	1,12	0.0006
	Rectrix saturation	-1.02	31.7	1,12	0.0005

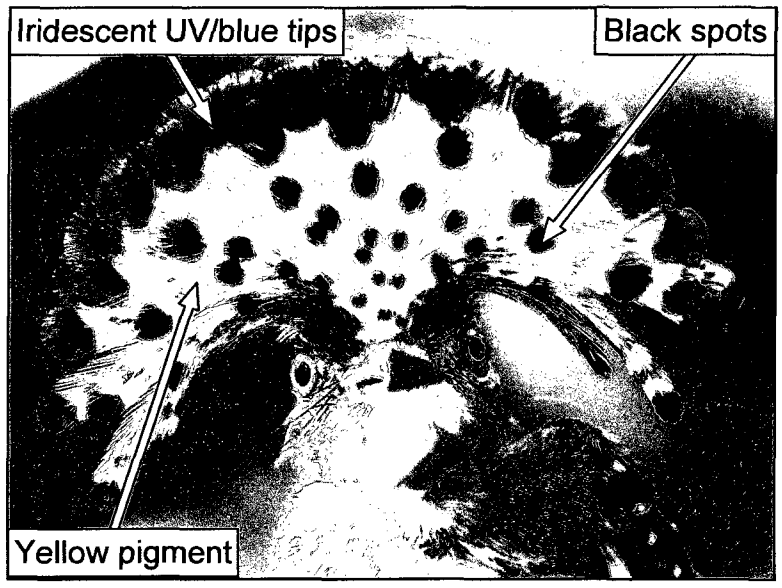

Figure 2.1. Female royal flycatcher showing fully erect crest.

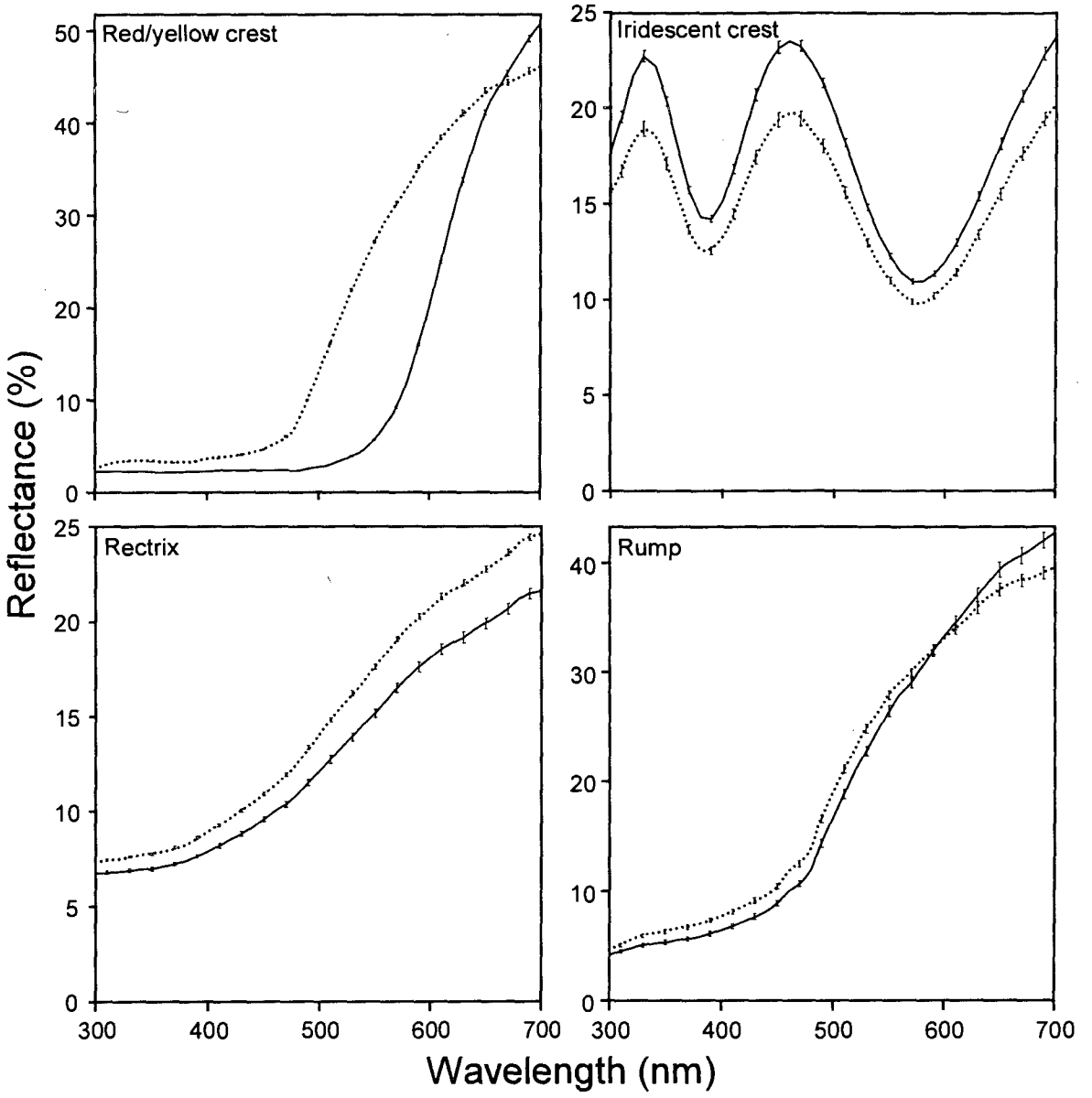

Figure 2.2. Average reflectance spectra for adult male (solid lines; n = 26) and female (dotted lines; n = 41) royal flycatchers. Vertical bars indicate standard errors. Note variation in y-axis scale.

CHAPTER 3: ELABORATE PLUMAGE PREDICTS REPRODUCTIVE SUCCESS IN MALE AND FEMALE ROYAL FLYCATCHERS BUT PAIRS DO NOT MATE ASSORTATIVELY: IMPLICATIONS FOR MUTUAL SEXUAL SELECTION

Synopsis

Sexual selection drives the evolution of elaborate traits in males through male-male competition and female mate choice. The theory of mutual sexual selection postulates that intrasexual aggression and mutual mate attraction may lead to the elaboration of traits in both sexes. To date, some studies of mutual mate choice have found patterns of assortative mating by sexual ornamentation and evidence that some female ornaments predict reproductive success, whereas other studies have failed to support mutual mate choice as a selective factor favouring the evolution of female ornamentation. The royal flycatcher (*Onychorhynchus coronatus*) is a Neotropical tyrant flycatcher that exhibits dual-sex ornamentation: both males and females display elaborate crest feathers and other colourful plumage. To investigate theories of mutual mate choice in this species, we evaluated the relationship between plumage colouration and reproductive success in both sexes, and examined whether royal flycatchers pair assortatively by plumage colouration, body size, or quality. We used reflectance spectrometry to quantify the brightness and saturation of four plumage regions, collected morphological and condition measurements, and quantified several measures of reproductive success for all adults in our population. We discovered that plumage colouration predicted several measures of reproductive success in both sexes. However, royal flycatchers did not appear to mate assortatively by ornamentation, morphology, or condition. To our knowledge, this is the first study to report this discordant pattern, and we provide several possible explanations for our results, including low mate encounter rates and confounding effects of natural selection on elaborate traits. Our study makes a

novel contribution to studies of mutual sexual selection, and suggests that multiple factors may interact to shape the evolution of elaborate traits in females.

Introduction

Studies of sexual selection have focused heavily the evolutionary basis for the widespread dyadic pattern of showy males and drab females. Sexual selection predicts that higher parental investment in one sex will lead to choosiness in that sex, driving stronger intrasexual competition for mating opportunities in the other sex (Trivers 1972, Johnstone et al. 1996). In many species, females invest more than males into offspring care and are consequently choosier about their choice of mates (Trivers 1972, Andersson 1994). This intensified selection on males often leads to the exaggeration of male traits that improve their success in competition with same-sex rivals or enhance their attractiveness to females (reviewed in Andersson 1994). Whereas sexual selection drives the evolution of elaborate male traits, natural selection is expected to drive female crypsis (Darwin 1871, Wallace 1891). However, across taxa we find many examples of exaggerated female ornaments, suggesting that natural selection is not the only force shaping the evolution of female traits (Amundsen 2000, Amundsen and Pärn 2006).

Multiple hypotheses attempt to explain why both sexes are ornamented in some species. The correlated response hypothesis suggests that females may exhibit ornaments that resemble those of males due to a genetic correlation between the sexes (Lande 1980). Since the majority of the male and female genome is shared within a species, female preferences for particular male traits could produce offspring (both male and female) that also bear the preferred trait. In this way, female preference for a male trait may drive the

expression of that trait in females, although the female trait may be entirely non-functional. This theory may clarify why, in some instances, exaggerated female traits are not preferred by males (reviewed in Amundsen 2000) and do not correlate with aspects of female quality (e.g. Hill 1993, Cuervo et al. 1996, Rohde et al. 1999). However, in some species, males prefer more ornamented females (reviewed in Amundsen 2000), and some female ornaments have been shown to signal quality (e.g., Jawor et al. 2004, Weiss 2006); therefore, we must consider alternatives to the correlated response hypothesis in some species.

One alternative is the mutual sexual selection hypothesis, which proposes that intrasexual competition may be strong in both sexes, and that both male and female preferences can drive the elaboration of traits in the opposite sex (Burley 1983, Parker 1983). Male choosiness may arise when males invest in offspring care, when there is a high female-to-male operational sex ratio, or when female quality varies greatly (Johnstone et al. 1996). More recent models also suggest that degree of parental investment, mate encounter rate, and costs of mate searching also play an important role in the evolution of mutual mate choice (Kokko and Monaghan 2001, Kokko and Johnstone 2002). Under these conditions (high male paternal investment, large pool of available potential mates, or high female quality variance), preferences may also lead to differential reproductive success if traits honestly reveal mate quality (reviewed in Andersson 1994).

Male mate choice for female traits can be inferred from the timing of pairing and egg laying, differential allocation by males, assortative mating, as well as observational and experimental studies of mate preference in the field or laboratory (Amundsen and

Pärn 2006). Although the latter two methods provide stronger inferential value, the vast majority of studies have focused on documenting patterns of assortative mating (reviewed in Amundsen and Pärn 2006). Assortative mating refers to the non-random pairing of males and females based on phenotype or quality (Burley 1983). There is evidence for positive assortative mating by body size or other morphometric traits in many taxa (e.g., Harari et al. 1999, McKaye 2006). In birds, while several studies found evidence for assortative mating by ornamentation (e.g., Andersson et al. 1998, Wagner 1999, Wiebe 2000, Jawor et al. 2003, MacDougall and Montgomerie 2003, Komdeur et al. 2005, Bitton et al. 2008, Bortolotti et al. 2008), other studies did not find such patterns (reviewed in Hill 2006). For instance, Murphy (2008) found no evidence for assortative mating by tail ornamentation, body size, or phenotypic condition in turquoise-browed motmots (*Eumomota superciliosa*), and Balenger et al. (2007) found no evidence for assortative mating by colour or body condition in the mountain bluebird (*Sialia currucoides*).

The assessment of assortative mating relative to colouration has been further complicated by the fact that although some studies have directly compared the same trait in both sexes (e.g. Andersson et al. 1998, Wiebe 2000, MacDougall and Montgomerie 2003), others have compared different male and female traits (e.g. Wiebe 2000, Jawor et al. 2003). In addition, while positive assortative mating may result from mutual mate choice, it may also derive from strong intrasexual competition, where intense male-male and female-female competition during the breeding season drives the pairing of the highest quality individuals (Creighton 2001), from the preferential mating of individuals' phenotypes along a continuum, where individuals of both sexes exhibit homotypic

preferences (Burley 1983), or through passive processes such as the exclusion of young, low quality individuals from high quality territories (Ferrer and Penteriani 2003). Moreover, mutual sexual selection does not necessarily lead to assortative mating, since different traits may be important in mate choice and intrasexual competition in males and females. Therefore, to document mutual sexual selection, studies of mutually ornamented species should also consider whether more elaborately ornamented individuals experience greater reproductive success (e.g., Berglund et al. 1997, Daunt et al. 2003, Velando et al. 2003).

In this study, we investigate the possibility that mutual sexual selection has favoured the evolution of elaborate plumage traits in both male and female royal flycatchers (*Onychorhynchus coronatus*). Royal flycatchers are small Neotropical passerines distributed from Mexico to South America (Fitzpatrick 2004). These birds are facultatively socially polygynous, with many males pairing monogamously, and some males pairing with two breeding females (Cuthbert and Doucet, unpubl. data). Females are solely responsible for nest-building and parental care in this species (Skutch 1960). Royal flycatchers exhibit striking dual-sex ornamentation: both males and females produce elaborate fan-shaped crests that they can display or conceal at will, conspicuous yellow rumps, and long, rufous tail feathers. In a recent study, we showed that plumage traits appear to signal different aspects of quality in both sexes, including nestling feeding rates in females and ectoparasite load and stress in males (Cuthbert and Doucet, in review). Those data suggest that both sexes might benefit from being choosy and using variation in plumage traits to make mate choice decisions. We therefore predicted that royal flycatchers should mate assortatively by plumage colour, condition, or morphology,

and that plumage colouration should correlate with reproductive success in both sexes. We tested these predictions by capturing mated pairs of free-living royal flycatchers, quantifying their plumage colouration using reflectance spectrometry, and monitoring their breeding attempts to determine apparent reproductive success. A recent study has shown that traits preferred repeatedly in laboratory mate choice experiments bear little significance to mate choice preferences in more natural conditions (Wilson et al. 2008), suggesting that, at the very least, observations of mating patterns in the wild are critically important in sexual selection studies.

Methods

We studied royal flycatchers in Sector Santa Rosa, Area de Conservacíon Guanacaste, Costa Rica. At this site, royal flycatchers begin to engage in breeding activities in late April, just before the onset of the rainy season. These birds usually build nests over rivers, and nests defended by different males were usually separated by more than 500 m. Our breeding population density was therefore very low, as has been reported in other subspecies of royal flycatcher (Fitzpatrick 2004), and finding active nests required extensive searching. Once we identified an active royal flycatcher nest, we used mist nets to capture the territorial male and female. We assigned a unique combination of coloured metal or plastic leg bands to each adult for subsequent visual identification. We also measured tarsus length, mass, wing chord, and crest length (from the base of the central crest feather to its tip), and collected at least five crest and rump feathers, as well as a single rectrix feather, from each individual. We used these feathers in our analyses of colouration (see below). We also measured several indicators of

condition in both sexes (details in Cuthbert and Doucet, in review). Briefly, we used the rectrix feather to analyse feather growth rate as an indicator of nutritional condition at the time of molt (reviewed in Grubb 2006). We counted the number of crest parasites present on the entire crest as a measure of ectoparasite load. We prepared a blood smear in the field from a small blood sample taken from the brachial vein to evaluate heterophil to lymphocyte ratio, a measure of immune stress (reviewed in Maxwell 1993).

We monitored active nests throughout two breeding seasons (May-August 2006 and 2007), collecting data on the number of active nests defended by a single male, lay date, the total number of eggs laid over the breeding period (clutch size is almost always two; Skutch 1960), the total number of nestlings produced by each pair over the breeding period, and the total number of nestlings fledged by each pair. It was important to monitor nests at each of these stages, since some clutches had fewer eggs (in some instances, we were unable to determine whether only a single egg was laid, or whether one egg was depredated), some eggs did not hatch or were depredated before reaching the nestling stage, and some nestlings were depredated before fledging. We also recorded predation events, and the number of nest attempts made by each pair throughout the season.

Reflectance spectrometry

Royal flycatchers develop elaborately coloured, elongated crest feathers, which they may conceal and display at will. Phaeomelanin pigments produce red colouration in males and yellow colouration in females (Cuthbert and Doucet, in review). The tips of crest feathers in both sexes are an iridescent UV-blue structural colour (Cuthbert and

Doucet, in review). When their crests are concealed, their most conspicuous plumage traits are their bright yellow rumps and rufous tails. The rest of their body plumage ranges from olive-brown (mantle) to buff beige (breast). In the lab, we mounted collected feathers onto matte black cardboard, overlapping the feathers from a given body region to approximate their placement on live birds. For the crest, we measured both the pigmented area of the crest responsible for red colouration in males and yellow colouration in females, and the iridescent UV/blue tips of the crest feathers. We measured colouration using a bifurcated fiber-optic probe, a reflectance spectrometer (USB4000, Ocean Optics, Dunedin, FL) and a Deuterium Tungsten Halogen light source (DH2000, Ocean Optics). A black rubber sheath was mounted on the probe to block external light and to maintain the probe at a fixed distance, perpendicular to the sample being measured. We collected five measurements for each region, each of which comprised an average of 20 measurements taken in rapid succession by the spectrometer operating software (OOIBase 32), and averaged these for our analyses. All reflectance data were collected relative to a Spectralon diffuse white standard (Labsphere, >97% reflectance).

We limited our analyses to the bird-visible portion of the reflectance spectrum (300-700 nm; reviewed in Cuthill 2006), and used CLR Colour Analysis Programs (Montgomerie 2008) to calculate the brightness and saturation of each region. We calculated brightness as the mean reflectance from 300 to 700 nm and saturation as the difference between the maximum and minimum reflectance divided by mean brightness. We did not calculate hue since our previous study showed that saturation and hue were highly positively correlated for all body regions in males and females ($P < 0.05$, Cuthbert and Doucet, in review).

Reproductive success and assortative mating

We investigated whether plumage colour related to reproductive success in both males and females. We identified mated pairs through nest observations of colour-banded individuals. Territorial males were routinely observed near the nest throughout the breeding season, chasing away conspecific males and heterospecific intruders, and displaying to the female. Territorial females were observed building nests, incubating eggs, and provisioning offspring. As with other investigations of sexual selection, as well as studies of mutual mate choice (reviewed in Amundsen and Pärn 2006), we used first egg date (reviewed in Verhulst and Nilsson 2008), clutch size (Lack 1947, 1948), hatching success (Mayfield 1975), and number of fledglings (Clutton-Brock 1988, Newton 1989) as measures of reproductive success. Through the regular monitoring of completed nests, we were able to determine first egg date for many of our nests, although if we did not find eggs on the day they were laid, we estimated lay date based on hatch date. When we did not witness fledging or document the survival of nestlings to fledging age, we considered those nestlings' fate to be unknown since depredation rates were high in both years of our study. We also included the number of nest attempts per breeding season as a measure of female breeding effort, since some females re-nested after a depredation event, while others abandoned the territory (Cuthbert and Doucet, unpubl. data). For all analyses of reproductive success, we calculated polygynous male totals based on data from both of his mates and used the first egg date from his primary female. We also compared the colouration of monogamous and polygynous males.

We also investigated whether royal flycatchers mate assortatively by colour, morphology, or condition. For instances where we had data from the same pair for both years, we included only data from the pair's first year in our analyses. For polygynous males, we included only the male paired to his primary female (determined by pairing date) in our analyses of assortative mating.

Statistical analyses

To investigate whether plumage colouration was related to reproductive success in royal flycatchers, we performed stepwise regressions with backward elimination, setting the probability to enter the model at 0.25, and the probability to leave at 0.10. We also performed Student's t-tests to examine whether colouration differed between monogamous and polygynous males. To investigate assortative mating, we performed Pearson correlations on our colourimetric, morphometric, and quality variables of interest for each pair. All statistical tests were performed using JMP, version 5.0 (SAS Institute Inc., Cary, NC).

Results

Reproductive success

Plumage colouration was a significant predictor of measures of reproductive success in both male and female royal flycatchers. In males, clutch size tended to be positively predicted by the brightness of the rump (Table 3.1). The number of nestlings was positively predicted by the saturation of the rectrix feathers, and negatively predicted by the brightness of the red crest and the saturation of the rump (Table 3.1). The number

of fledglings was negatively predicted by the brightness of the iridescent crest and the saturation of the rump (Table 3.1). The total number of nest attempts per breeding season was positively predicted by male rump brightness (Table 3.1). First egg date was not predicted by any male colourimetric variables (Table 3.1). Polygynous males had brighter rumps than socially monogamous males (Fig. 3.1; $t_{16} = -2.74$, $P = 0.015$). No other colorimetric variable was related to male polygyny.

In females, the number of nestlings per breeding season was significantly positively predicted by the saturation of the rectrix feathers. We also discovered a number of trends between a female's plumage colour and her reproductive success. For example, the number of nestlings was predicted by the brightness of the iridescent crest feathers (Table 3.2). The number of nest attempts per season was positively predicted by the saturation of the iridescent crest feathers (Table 3.2). First egg date was positively predicted by the brightness of the rectrix and the saturation of the rump, and negatively predicted by the brightness of the iridescent crest,Table 3.2). Clutch size and number of offspring fledged were not predicted by any female colourimetric variables (Table 3.2).

Assortative mating

We found no evidence of assortative mating by colouration, morphology, or condition in royal flycatchers. For plumage colouration, only the saturation of the rectrix ($r = 0.40$, $P = 0.14$, $N = 13$) and pigmented crest feathers ($r = -0.37$, $P = 0.17$, $N = 13$) approached significance (all other $P > 0.27$). For morphological measurements, only wing chord approached significance ($r = 0.41$, $P = 0.13$, $N = 13$; all other $P > 0.61$). For condition indicators, male and female feather growth rates were not correlated ($r = 0.02$,

$P = 0.93$, $N = 13$), nor was there a correlation between the sexes for heterophil to lymphocyte ratio, ($r = -0.41$, $P = 0.18$, $N = 10$) or crest parasite load ($r = 0.12$, $P = 0.69$, $N = 12$). Even when we used a less conservative approach by including all pairs from both years in our analyses ($N = 22$), there was no evidence of assortative mating by any of these traits (all $P > 0.07$).

Discussion

Mutual mate choice is expected to occur in species where both males and females stand to gain significant benefits from being choosy (Johnstone et al. 1996). This choosiness may drive the elaboration of traits in both sexes, if more ornamented individuals of both sexes are preferred and consequently achieve higher reproductive success. In male royal flycatchers, plumage colouration predicted reproductive success and number of mates. Males with more saturated rectrices and brighter rumps had higher reproductive success through higher numbers of nestlings, fledglings, mates, and number of re-nest attempts by their mate throughout the breeding season. We also found that males with darker, more pigmented crests tended to produce more nestlings than lighter males.

In this species, red crest colouration, and probably the colouration of other plumage regions, is produced by the deposition of phaeomelanin pigments (Cuthbert and Doucet, in review). Although the pigmentation of the crest is highly saturated, rump pigmentation is very faint, and rump colouration may instead be selected to contrast brightly against the dark surrounding feathers, perhaps through modification of the underlying microstructure rather than the pigments themselves, as has been shown for

carotenoid pigmentation (Shawkey and Hill 2005). Our previous study showed that male plumage colouration was correlated with indices of health and condition in this species (Cuthbert and Doucet, in review). Phaeomelanin colouration has also been shown to be dependent on stress levels in barn owls (*Tyto alba,* Roulin et al. 2008), and may emerge as another honest indicator of quality (Jawor and Breitwisch 2003, McGraw 2005). Taken together, our findings suggest that plumage colouration is a sexually-selected signal of quality in male royal flycatchers. Because male investment in reproduction is restricted to sporadic territory and nest defence, females may assess male traits to gain information about indirect genetic benefits rather than direct benefits (Kirkpatrick 1996, Kirkpatrick and Barton 1997, Cameron et al. 2003).

Having shown that plumage colour predicted reproductive success in male royal flycatchers, we were interested in determining whether elaborate traits might also predict female reproductive success in this species. We found that females with more saturated rectrix feathers produced more nestlings in a breeding season. We also uncovered trends for many other plumage variables predicting female reproductive success; the brightness of the iridescent crest predicted the number of eggs to reach the nestling stage per breeding season and first egg date, such that females with brighter iridescent crest tips laid eggs earlier, yet had fewer nestlings than females with dull tips. Iridescent crest saturation also predicted the number of nesting attempts per season, such that females with more saturated crest tips were more likely to re-nest followed depredation. Interestingly, iridescent crest brightness also predicted nestling feeding rates by females in a previous study of this same population (Cuthbert and Doucet, in review). Females with darker, more pigmented rectrices but less saturated rumps also tended to lay eggs

earlier in the season. Although these data suggest that female colouration may be sexually selected in this species, some of the colour variables predicted reproductive success and condition in opposing directions. For instance, females with bright iridescent crest tips fed offspring less often, laid their eggs earlier in the season and had fewer nestlings in a season, however females with more saturated crest tips were more likely to re-nest following depredation. Females with less pigmented rumps provisioned nestlings more often and laid eggs earlier, yet were more stressed (Cuthbert and Doucet, in review). Perhaps different plumage traits trade-off against one another. Alternatively, plumage colouration may represent a measure of female breeding effort rather than a sexually-selected signal of quality. To date, studies have reported mixed results for the relationship between female colouration and reproductive success. In female eastern bluebirds (*Sialia sialis*), structural plumage colour predicted lay date (Siefferman and Hill 2005), and in female North American barn swallows (*Hirundo rustica*), phaeomelanin-based breast and belly colouration predicted lay date, number of offspring fledged, and number of nesting attempts during the breeding season (Safran and McGraw 2004). In contrast, a study of bluethroats (*Luscinia svecica*) revealed no correlations between female plumage colouration and clutch size, hatch date, nestling feeding rate, or nestling growth (Rohde et al. 1999).

Since plumage colouration reveals quality and correlates with reproductive success in both male and female royal flycatchers, it seems logical to infer that elaborate ornaments are sexually selected indicators of quality in both sexes. Such sexually selected traits could evolve through intrasexual competition or mate choice (Darwin 1871). Patterns of positive assortative mating arise when either intrasexual competition or

direct mate choice drive the pairing of the highest quality, most elaborately ornamented individuals. Mutual preferences may arise from homotypic preferences (Burley 1983), the correlated evolution of traits and preferences (Lande 1980, Halliday and Arnold 1987, Servedio and Lande 2006), or from either Fisherian or honest indicator models of sexual selection (Andersson 1994). It is important to acknowledge that whichever the origin of mate choice in a species, the same pattern of assortative mating will arise when mutual preferences influence pair formation. If either competition or mate choice was a driving factor in royal flycatcher pair formation, we might expect to find a pattern of positive assortative mating by plumage colouration, where the most colourful males would be mated to the most colourful females. However, we did not find evidence for positive assortative mating for plumage colouration, nor did we find evidence of assortative mating by body size or condition.

There are a number of possible explanations for the apparent lack of assortative mating in royal flycatchers. One possibility is that assortative mating does exist in this population but that we failed to detect it. First, because of a low population density of royal flycatchers (a total of 14 nesting attempts in 2006 and 19 in 2007 in an area >10 km^2) and the presence of some of the same individuals in both years, the sample sizes were small for some analyses (N= 13 breeding pairs). Other studies of assortative mating have detected significant patterns with slightly larger sample sizes (18-22 pairs, e.g., Andersson et al. 1998, MacDougall and Montgomerie 2003), although even our less conservative analysis with a larger sample size (N= 22) did not reveal any obvious patterns of assortative mating. Another possibility is that in phenotypically mismatched pairs, the more attractive (and presumably higher quality) member of the pair may seek

out extra-pair copulations (EPC) (Petrie and Hunter 1993). We were unable to determine the presence of extra-pair offspring, but if these birds engage in EPCs, there could be a pattern for assortative mating through cryptic female choice for more ornamented extra-pair mates. Although genetic mating systems have been characterized in only a few tropical species, with the percentage of extra-pair young varying from 0 % (Fleischer et al. 1997) to 50 % (Carvalho et al. 2006), extra-pair matings are thought to be less common in tropical species (Stutchbury and Morton 2001). In royal flycatchers, we regularly capture or encounter males other than the territorial male on territories, and these males may be seeking to copulate with territorial females. Extra-pair fertilizations are also expected in species which do not exhibit paternal care (reviewed in Griffith et al. 2002), and if they are common in royal flycatchers, they may explain a lack of assortative mating by colouration in social pairs this species.

Alternatively, if our study accurately reflects a lack of assortative mating in the royal flycatcher, then this pattern could be explained by a number of non-mutually exclusive mechanisms. First, as implied earlier, intrasexual selection could be more important in driving ornament elaboration in one or both sexes, and this pattern would be consistent with our documented associations between colouration, quality, and reproductive success in both sexes. A second possibility is that the different sexes prefer different traits, which might lead to mutual mate choice by different combinations of traits. The fact that different traits predicted reproductive success in males and females indirectly supports this suggestion. Third, because this species nearly always builds its nests over rivers and seems to use rivers as movement corridors, individuals are separated by long linear distances. These observations suggest that potential mate encounter rates in

this population are very small, which may lead birds to be less choosy, and to accept a mate without fully evaluating that individual against others in the population (Kokko and Monaghan 2001, Kokko and Johnstone 2002). Fourth, it is also possible that quality does not vary sufficiently across individuals to warrant the costs of being highly discriminatory during mate choice (Johnstone et al. 1996). Fifth, it is possible that our small population of royal flycatchers in Santa Rosa National Park, which is isolated from other populations by wide expanses of unsuitable habitat, is relatively inbred. In such a population, choosing a mate based on genetic dissimilarity rather than elaborate ornaments may be more important, to reduce inbreeding depression (Tregenza and Wedell 2000, Mays and Hill 2004, Neff and Pitcher 2005). A final possibility is that patterns of mate choice for particular traits could be confounded by natural selection on those traits. For example, in mutually ornamented turquoise-browed motmots, exaggerated tail ornaments are correlated with reproductive success in males but not females (Murphy 2007), and birds do not pair assortatively by tail characteristics (Murphy 2008), but elaborate tails appear to function as naturally-selected pursuit deterrent signals in both sexes (Murphy 2006). In royal flycatchers, elaborate crests and accompanying movements have been proposed to function as a startle display in defense against predators (Graves 1990), and birds continuously perform this display to potential predators (humans) while being captured and handled (Cuthbert and Doucet, pers. obs.). Thus, natural selection for particular shapes and colours that produce more effective startle displays might mask potential mate choice patterns for slightly different crest characteristics.

Our study contributes to a growing body of literature on mutual sexual selection in mutually ornamented species. Our findings suggest that colourful plumage could have evolved as a sexually selected indicator of quality in both sexes of the royal flycatcher. Our study also emphasizes the importance of examining multiple candidate traits when investigating reproductive success; though royal flycatcher crests are arguably their most notable visual ornament, we also documented important relationships between reproductive success and other plumage traits in both males and females. Finally, our findings emphasize the fact that assortative mating is not a prerequisite for mutual sexual selection and the evolution of mutual ornamentation – preferences for different traits, intrasexual selection, and natural selection may also play important roles, and the latter may be particularly important species where elaborate traits appear to function as both sexual ornaments and antipredator displays.

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Table 3.1. Significant predictors of reproductive success in adult male royal flycatchers using stepwise regressions with a backward elimination procedure (see Methods for details).

Dependent variable	Model predictors	R^2 or β'	F	df	P
First egg date	No significant predictors				> 0.16
Clutch size	Whole model	0.21	3.89	1, 15	0.07
	Rump brightness	0.45	3.89	1, 15	0.07
Number of nestlings	Whole model	0.54	5.54	3, 14	0.01
	Red crest brightness	-0.32	2.23	1, 16	0.16
	Rump saturation	-0.62	9.56	1, 16	0.008
	Rectrix saturation	0.43	4.76	1, 16	0.05
Number of fledglings	Whole model	0.61	8.58	2, 11	0.006
	Iridescent crest brightness	-0.50	6.69	1, 12	0.03
	Rump saturation	-0.50	6.74	1, 12	0.02
Number of nest attempts	Whole model	0.31	7.20	1, 16	0.02
	Rump brightness	0.56	7.20	1, 16	0.02

Table 3.2. Significant predictors of reproductive success in female royal flycatchers using stepwise regressions with a backward elimination procedure (see Methods for details).

Dependent variable	Model predictors	R^2 or β'	*F*	df	*P*
First egg date	Whole model	0.26	2.34	3, 20	0.10
	Iridescent crest brightness	-0.37	3.65	1, 22	0.07
	Rectrix brightness	0.36	2.99	1, 22	0.10
	Rump saturation	0.39	3.45	1, 22	0.08
Clutch size	No significant predictors				> 0.13
Number of nestlings	Whole model	0.28	4.75	2, 24	0.02
	Iridescent crest brightness	-0.31	3.13	1, 25	0.09
	Rectrix saturation	0.41	5.69	1, 25	0.03
Number of fledglings	No significant predictors				> 0.26
Number of nest attempts	Whole model	0.13	3.87	1, 26	0.06
	Iridescent crest saturation	0.36	3.87	1, 26	0.06

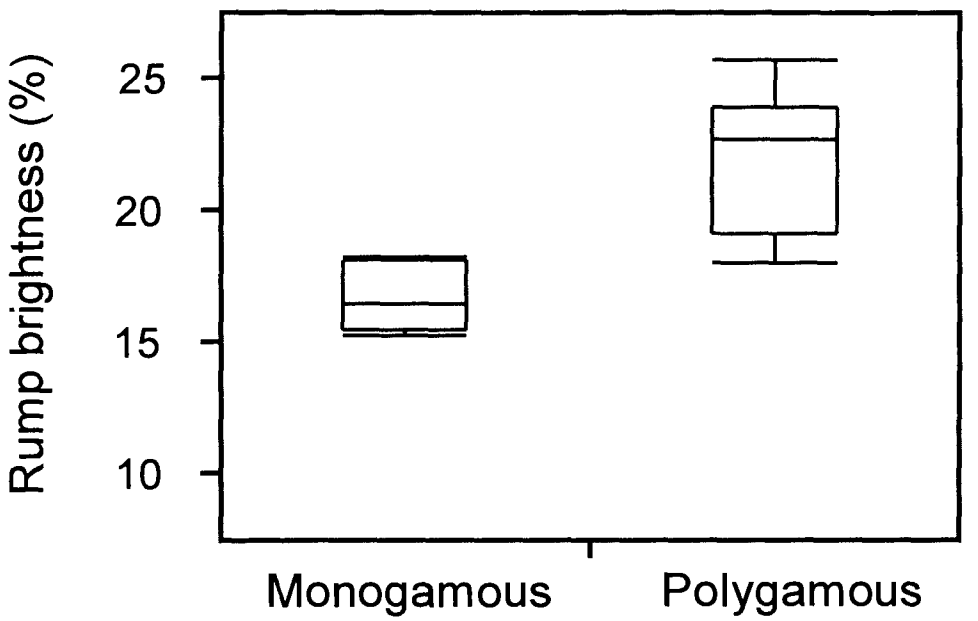

Figure 3.1. Polygynous male royal flycatchers have brighter rumps than monogamous males ($t_{16} = -2.74$, $P = 0.015$). Box plots show 10th, 25th, 50th, 75th and 90th percentiles with horizontal lines.

Thesis Summary

While sexual selection theory has traditionally focused on the widespread occurrence of showy males and inconspicuous females, less attention has been given to the evolution of female ornamentation. Females of many species exhibit showy traits, and several hypotheses attempt to explain this phenomenon. Among them, the theory of mutual sexual selection posits that elaborate ornaments evolve in both sexes via mate choice and/or intrasexual competition. We investigated the signal function of plumage colouration in royal flycatchers, which exhibit elaborate mutual ornamentation. Royal flycatchers produce large, fan-shaped crests which are red in males, and yellow in females, both with iridescent tips. Royal flycatchers also have buff-yellow rumps and rufous tail feathers.

In Chapter 2, we investigated differences in plumage colouration in males and females, and whether these traits were condition-dependent. We discovered extensive sexual dimorphism and sexual dichromatism in royal flycatchers, and documented delayed plumage maturation in males. We also found that both male and female plumage colouration signal aspects of individual quality. We conclude that plumage colouration is indeed an honest signal of quality in royal flycatchers, though quality indicators differ between the sexes, and among plumage patches.

In Chapter 3, we investigated whether or not royal flycatcher plumage colouration predicts reproductive success, and whether pairs mate assortatively by degree of ornamentation. Although we did find that plumage colouration predicted reproductive success in both sexes, intriguingly we did not find evidence for assortative mating by plumage colouration, body size, or quality in this species. We suggest that plumage

colouration is a sexually-selected trait in both male and female royal flycatchers, and that the lack of assortative mating does not necessarily reflect a lack of mutual mate choice in this species.

Overall, it appears that both male and female royal flycatchers exhibit condition-dependent plumage colouration which predicts reproductive success. These findings suggest that plumage colouration is important to mate choice in this species. We propose that the evolution of female ornamentation in royal flycatchers has been shaped by several factors, including mutual preferences for different plumage traits, intrasexual selection, and natural selection.

Our study is the first to quantify plumage colouration in royal flycatchers, and to extensively study their breeding behaviour in a colour-banded population. We provide many insights into the function of royal flycatcher plumage colouration, and suggest many avenues for further research in this dynamic species. We also make significant contributions to the study of mutually ornamented species, emphasizing that assortative mating is not a prerequisite for mutual mate choice, and that many factors may influence the evolution of female ornamentation.

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